8-2013

The Use of Non-Traditional Technologies to Improve the Efficiency and Sustainability of Modern Poultry Production

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A dissertation submitted in partial fulfillment
of the requirements for the degree of
Doctor of Philosophy in Poultry Science

by

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Bachelor of Science in Biological and Agricultural Engineering, 2002

August 2013
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This dissertation is approved for recommendation to the Graduate Council.

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ABSTRACT

Historically, major driving factors for the animal agriculture industry have been efficiency and profitability. As demand for efficient food production has increased, the industry has focused research efforts on ways to improve the rearing process. Current market demands are requiring the industry to abandon some of the traditional tools it has used to maximize productivity. However, developing alternative technologies are available which may fill the void. Unfortunately, these alternatives are less well-described and the beneficial impacts they can have are not fully understood. As the animal agriculture industry matures it is becoming evident that consumers will continue to demand methods of production change to increase sustainability, produce safer food, produce food that is perceived to be more natural, and improve welfare of animals. In order to maintain profitability, companies have been very responsive to market pressures. As customers demand a particular product, companies make efforts to fulfill demand, or risk losing market share. The goal of the studies included herein is to determine what impact selected socially acceptable, non-traditional technologies can have on the efficiencies of poultry production. The first study evaluates suitability of a commercially available direct-fed microbial (DFM) to replace traditional antibiotic growth promoters (AGP) in broiler feeds. This study indicates that an effective DFM can replace traditional AGP in poultry feeds and also improve growth efficiencies of poultry currently being grown without AGP. A second study evaluates the effects of inclusion of a unique blend of organic acids in the drinking water of turkeys on body weight loss during feed withdrawal and transport periods. The study suggests that by inclusion of this specific organic acid blend in the drinking water prior to harvest, body weight was positively affected in a manner that meaningfully impacts profitability. Taken together, these

studies present non-traditional alternatives for implementation by poultry producers in an effort to meet consumer demands, improve welfare, and increase profitability.

DEDICATION

I would like to dedicate this work to my family. To my mother who always did what she had to so I could do what I wanted, my father for continuously reminding me why college was the option I wanted to take in life, my wife for her continuous support and love through the years, and my daughter for motivating me to be an example worthy of her admiration.

ACKNOWLEDGEMENTS

I would like to take this opportunity to thank the members of my committee. Your support and guidance enabled me to fulfill a dream that was many years in the making. Thank you, Lisa, Luc, Memo, and Walter. I would also like to thank you, Billy. The “opportunities” you have given me over the last few years have shaped my life in a profound way. Looking back, it is hard to believe that working with you enabled me to take my very first trip out of the USA. Since that time we have been around the world together. I have had the chance to experience what Mark Twain so eloquently stated when he said:

> *“Travel is fatal to prejudice, bigotry, and narrow-mindedness, and many of our people need it sorely on these accounts. Broad, wholesome, charitable views of men and things cannot be acquired by vegetating in one little corner of the earth all one's lifetime.”*

There are those that might argue I still require a good deal more travel, but I am certainly not the person today that I was in 2002 when we first started working together.

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LIST OF ABBREVIATIONS

ADG	average daily gain
ANOVA	analysis of variance
ATP	adenosine tri-phosphate
BMD	bacitracin methylene disalicylate
BW	body weight
CE	competitive exclusion
cfu	colony forming units
d	day
DFM	direct-fed microbial
EO	essential oil
EU	European Union
FAO	Food and Agricultural Organization of the United Nations
FCR	feed conversion ratio
FDA	United States Food and Drug Administration
FOS	fructooligosaccharide
FW	feed withdrawal
g	gram
GALT	gut associated lymphoid tissue
GIT	gastrointestinal tract
GLM	general linear model
h	hour
kg	kilogram
MIC	minimum inhibitory concentration
min	minute
NSP	non-starch polysaccharides
OA	organic acid
USDA	United States Department of Agriculture
VRE	Vancomycin-resistant *Enterococci*

LIST OF PAPERS

Chapter III:
Pixley, C.M., B.M. Hargis, and R.E. Wolfenden. 2013. Evaluation of Direct-Fed Microbials as Alternatives to Antibiotic Growth Promoters in Modern Poultry Production. Prepared for Submission in The Journal of Applied Poultry Research 2013

Chapter IV:
Pixley, C.M., J. Barton, J.L. Vicente, A.D. Wolfenden, B.M. Hargis and G. Tellez. 2010. Evaluation of a Commercially Available Organic Acid Product During Feed Withdrawal and its Relation to Carcass Shrink in Commercial Turkeys. International Journal of Poultry Science: 9 (6): 508-510.

I. INTRODUCTION

Recent trends in the animal agriculture industry have been transitioning to production methods which are perceived to be more natural and sustainable. These trends are primarily shaped by social pressures from consumers in the general public. As a result, the industry is looking for ways to reduce or eliminate the inclusion of chemicals and compounds the general public has begun to demand be removed from production practices. Antibiotic growth promoters (AGP), in particular, have been singled out due to the emergence of antibiotic resistant bacteria, its apparent association with food animals[1], and the prevalence of negative press regarding these organisms in popular media. While a driving force behind the movement is the consumers of animal protein, in some cases government organizations have enacted bans on inclusion of AGP. In 2006, a full ban on the use of AGP was enacted in the European Union[2] and in 2011 South Korea followed suit[3]. While these two cases are significant, the overall motivating factor to industry has been consumer demand and the response of food providers to consumer trends. Many popular restaurant chain companies such as McDonald's, Kentucky Fried Chicken, Wendy's, Hardees, Subway and Chipotle have required suppliers to guarantee meat they purchase has been grown without the use of AGP[4]. As a result of this demand, the animal agriculture industry has been forced to re-evaluate production methods and search for sustainable alternatives.

In addition to a consumer driven shift in how poultry are produced, a major upcoming challenge the poultry industry will face is the projected increase in demand for product in the next 40 years. Current projections suggest that by 2050 demand for food in the world will increase by 100%[5,6]. Industries providing high value animal protein will be under extreme pressure to meet rising needs of a global middle class projected to grow by 3 billion people[7,8].

Asia's middle class is growing most rapidly and is set to represent 66% of the global middle class population and account for 59% of middle class consumption by 2030, up from its current rates of 28% and 23%, respectively[7]. These emerging middle class markets will be the primary drivers for increased demand for animal proteins, as diet is one of the first areas to improve as income levels rise[8,9]. Poultry is often a protein of choice for emerging middle class consumers, and it is predicted to grow the fastest of all animal agriculture sectors[8].

Such a large projected growth in consumption in combination with restrictions on modern production methods, such as removal of AGP, will require the poultry industry to take a forward look and determine the most efficient way to supply the market demands. Serious consideration and scientific effort needs to occur to determine the best path forward. The objective of the enclosed body of work is to determine what impact the use of non-traditional technologies might have on modern poultry production. These technologies would need to meet the criteria of a discerning market that is focused on removal of chemotherapy and improving animal welfare practices of the animal agriculture business. In order to be widely accepted, these technologies would need to improve the efficiency and costs associated with raising poultry.

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II. LITERATURE REVIEW

History of the Poultry Industry

Over the last century, the poultry industry has grown from a locally oriented businesses into a highly efficient, vertically integrated, progressive industry supplying product to a global market[1, 2]. The poultry industry, unlike many other animal agriculture industries, is highly structured with integrated control. From eggs to packaged food, the entire process is often controlled by a single company, including the feed which is consumed by the animals.

Prior to the 1920s the majority of poultry were backyard flocks intended to supply eggs and meat for a family with a few larger flocks that would supply eggs and meat locally[1,3]. Poultry products were only available seasonally due to limited knowledge of poultry physiology and nutrition. In the 1920s, demand for eggs increased resulting in an excess of male chickens[3]. These male chickens were fed and sold for meat, becoming the earliest form of the modern broiler[3]. It was observed that some of these chickens grew more rapidly than others, and some were better suited for producing eggs[3]. These observations led to the earliest work in poultry genetics, which resulted in development of the commercial birds used today[4]. The poultry industry, as we would recognize today, began taking shape in the 1940s. Until 1942 meat chickens were typically sold “New York dressed”, with only the blood and feathers removed, until an Illinois plant was the first to win government approval of “on-line” evisceration[1]. It was also during the 1940s that the first integrated operations began taking shape, consolidating feed mills, hatcheries and processing plants[1,3]. By the 1960s greater than 90% of poultry produced in the United States came from integrated poultry companies[1,3]. In 1949 the United States Department of Agriculture (USDA) established the first quality standards for processed poultry, and in 1959 federal inspection became mandatory[1,3]. By the 1970s, the poultry industry closely resembled todays', being highly automated, mechanized, utilizing specially selected genetic lines, and implementing strict nutritional and disease control programs[1,3]. The ability of the industry to provide economically priced, desirable packaged food to consumers resulted in chicken surpassing pork consumption in 1985 and beef by 1992 to become the most popular animal protein in the United States[1,3]. USDA 2010 statistics list the broiler market in the United States alone as worth more than $45 billion, providing more than 36 billion pounds of meat annually[5]. In order to meet high consumer demand in a market with low profit margins, poultry are reared in large densely packed houses typically containing greater than 10,000 birds, making a single flock worth thousands of dollars, in many instances over $100,000 on farms containing multiple houses, thus making control of disease a top priority[5,6].

Enteric Disease

Disease can be defined as any deviation or interruption of the normal structure or function of any part, organ or system within the host[7]. Enteric disease refers to disease of the gastrointestinal tract (GIT)[7]. In poultry production enteric disease results in the loss of productivity, an increase in mortality, and an increase in the potential for human health risks associated with food borne illness[8]. All of these contribute to increases in the cost associated with poultry production[8]. Digestion and absorption of nutrients, primary roles of the GIT, are affected by transit time of ingesta, pH, and changes in net absorption of water[7]. The GIT also acts as a barrier, protecting internal organs from exposure to pathogens found in the lumen. The GIT represents the largest mass of lymphoid tissue in the body, known as the gut associated lymphoid tissue (GALT)[9]. Pathogens are forced to contend with many natural defenses of the host to cause disease. Low gastric pH, rapid transit through portions of the GIT, competitive intestinal microbiota, and GALT all act in an effort to prevent pathogens from causing disease. In addition to insult from pathogens (bacterial, viral, fungal, parasitic), enteric disease can be caused by nutritional factors, stress, injury, and ingestion of toxins. These factors, if not causing disease outright, can leave a host more susceptible to disease[10]. The severity and duration of stress factors, such as suboptimal temperatures, poor environmental conditions, and improper handling can also influence susceptibility to disease[10].

Normal Microflora of the Gastrointestinal Tract

The GIT of warm blooded vertebrates constitutes one of the most diverse and densely populated ecosystems known. The number of organisms present in the GIT exceed the number of cells in the body by a factor greater than 10[11,12], and total microbial content of the GIT has been estimated to contain 10^{13}-10^{14} organisms[12,13]. Since the incorporation of molecular techniques, research indicates greater than 500 species of bacteria are present in the GIT, the majority of which have not been cultured[13,14]. The GIT of poultry is often anatomically split into the ileum and cecum. Lu *et al* found that *Lactobacillus* was the predominate genus of bacteria in the ileum and 65% of cecal microflora were of the *Clostridiaceae* family[13]. The microbial ecology of the poultry GIT has been shown to vary according to bird type, diet, age, and by anatomical region[13,15,16]. Despite the variability the predominant organisms in the GIT of poultry have been shown to be Gram positive anaerobes[13,16]. The majority of microflora present in the GIT are beneficial to the host by providing nutrients through their own digestive process and restricting the growth of pathogens, as discussed in more detail below[15,17]. Evidence also indicates gut microflora modulate enteric immune function[18], and are involved in maturation of the immune system[19]. Full understanding of the complex role the microflora in the GIT play is not yet fully understood.

History of AGP in Poultry Production

The term "antibiotic" was first used by Selman Waksman in reference to antagonistic substances with the capability to inhibit or kill other bacteria and fungi[20]. The most famous antibiotic to be characterized is probably penicillin, discovered by Sir Alexander Fleming in 1928 for which he won the Nobel Prize in Physiology or Medicine in 1945[21]. Fleming's work is said to be the inspiration for Waksman's research, which resulted in the discovery of streptomycin, for which he was awarded a Nobel prize in 1952[22]. The ability of antibiotics to increase growth rates in experimental animals was first observed in 1942 by Black *et al*[23], and Nielsen and Elvehjem[24]. In 1946 Moore *et al* first reported an improvement in the growth rate of poultry with the inclusion of an antibiotic in studies attempting to determine baseline vitamin requirements of poultry by eliminating microbial interference[25]. Moore observed that contrary to the hypothesis that antibiotics would sterilize the intestine, the total number of recoverable organisms did not change[25]. Although the drugs failed to create sterile conditions in the intestinal tract, sulfasuxidine and streptomycin, given in combination with additional dietary folic acid, reversed the negative growth effects produced by administering these drugs alone as well as the negative effects in chicks given only folic acid[25]. It was also observed that by inclusion of antibiotics and supplementary folic acid in the diet the predominate organism in the feces shifted from coliforms to *Lactobacilli*[25]. This observation may give key insight into why performance enhancement associated with AGP often is very similar in both frequency and magnitude to those observed with probiotic administration.

This research was part of a greater effort occurring in the 1940s, which resulted in the development of modern poultry production. Focused research was responsible for significant advancements in nutrition, genetics, management and marketing during this time period[26,27].

These advancements established the poultry industry as a large scale, highly efficient system to provide food to a growing market[1-3]. It also led to new challenges associated with nutrition and disease. It was during this time that much of our current understanding of poultry production was developed. As demand and availability for animal feed ingredients changed, new sources of feed ingredients were evaluated. In a series of evaluations, research indicated that incorporation of dried mycelia of the fungus *Streptomyces aureofaciens* acted as a growth promoter in poultry, later it was discovered that these dried mycelia contained the antibiotic chlorotetracycline[28,29,30,31,26]. The United States Food and Drug Administration (FDA) approved use of antibiotics as animal feed additives without the need for a veterinary prescription in 1951[26].

Following the approval of antibiotics for use as non-prescription feed additives a great deal of experimental data documenting the impact of inclusion of various antibiotics on body weight was generated. Heth and Bird published a summary of trials occurring from 1950-1953 which report 8.5% increased BW in chickens fed diets containing 4 to 35 mg/kg procaine penicillin, and improvements of 8.8% in experiments occurring between 1956 and 1960[32]. Increases in growth rate of 12.3% were observed in chickens consuming diets containing 10 to 35 mg/kg of tetracycline from 1950-1953, and 10.2% in experiments from 1956-1960[32]. From 1956 to 1959, 10 to 35 mg/kg zinc bacitracin improved growth by 6%, and 100 mg by 15%[32]. In 1980 Bird summarized data from multiple investigators examining the growth promoting effects of antibiotics between 1968 and 1980[33]. These data indicated that feeding penicillin resulted in an average BW increase of 11%, tetracyclines increased BW by 8-10%, and 4-7% average increase in BW for “new” antibiotics was observed[33]. “New” antibiotics referred to lincomycin, bambermycin, and tylosin[33]. Dafwang *et al* reported similar results in a series of studies conducted between 1981 and 1982 when they used the maximum approved rate of application for penicillin, oxytetracycline, lincomycin, bambermycin, and tylosin.[34]. Average BW improvement of birds consuming feed containing penicillin was reported as 10.4%, 11.5% for diets containing oxytetracycline, 10% for diets containing lincomycin, 14.3% for bambermycin containing diets, and 18.5% for diets containing tylosin. The number of reports showing positive growth response to AGP promoted their universal adoption by modern animal agriculture. Unlike therapeutic administration, an AGP is administered at sub-therapeutic dosages for a prolonged period of time with the intent of improving growth rates, meat quality, and efficiency in animals intended for food production.

In more recent studies, data suggests the positive effects of AGP have become less profound[35]. This trend is hypothesized to be a result of further improvements in management, genetics, and facilities[35]. Opponents to the use of AGP argue that the practice increases the risk of endemic bacterial populations developing resistance to the antibiotics, which is one potential hypothesis for the apparent the loss of efficacy of these compounds. As early as 1954 there was evidence indicating that consistently feeding an AGP for several years could result in loss of its ability to have a positive growth effect[36,37]. This loss of efficacy was shown to be reversible by the substitution of a different AGP, indicating the problem might be associated with development of resistance[38]. Indications of emerging antimicrobial resistance in animals intended for food began as early as 1951 with turkeys being fed streptomycin[39]. Similarly, resistance was observed in chickens fed tetracycline as an AGP in 1958 and 1959[22].

Currently, there are 32 antimicrobial compounds approved by the FDA for use in broiler feeds without a veterinary prescription, 15 are listed for treatment of coccidiosis, 11 are listed as growth promoters, and six are listed for other purposes[26]. Seven of these compounds (bacitracin, chlortetracycline, erythromycin, lincomycin, novobiocin, oxytetracycline, and penicillin) are used in both animal feeds and human medicine[26]. The public debate about the prudence of using antibiotics as growth promoters has been well documented, and both sides of the debate have extensive scientific data to support their arguments. Unfortunately there is little unbiased data available on the risks associated with AGP usage[26]. There is clear data supporting the emergence of resistance to an antibiotic in a population after it is commonly used for a period of time, and this has been well documented in poultry[39,40]. The argument has centered on the impact of these resistances and if they are transferred between human and animal populations. One of the main points of justification for proponents of AGP centers on Avoparcin[41], a glycopeptide structurally related to vancomycin and teicoplanin, that was widely used in Europe as a growth promoter from the early 1970s until it was fully banned in 1997[41]. Avoparcin was never used as an AGP in the United States[42]. Vancomycin-resistant *Enterococci* (VRE) were first reported in the mid-1980s and are now an important cause of nosocomial infections[43,44,45,46]. The fact that VRE are found in both Europe and the USA is used to argue that antimicrobial resistance is not a problem resulting from AGP usage, rather, it is a result of therapeutic use, primarily in human medicine[47]. Supporters of AGP have held to the belief that while resistance has developed to antibiotics used as AGP, removal of AGP would result in a greater risks to human and animal health than what is currently attributed to AGP use[47,48]. Antibiotic growth promotors have important prophylactic activity and their withdrawal is now associated with a deterioration in animal health, including increased diarrhea, weight loss and mortality due to *Escherichia coli* and *Lawsonia intracellularis* in early post-weaning pigs, and clostridial necrotic enteritis in broilers[47]. A directly attributable effect of these infections is the increase in usage of therapeutic antibiotics in diseased animals, including those of importance in human medicine such as tetracycline, aminoglycosides, trimethoprim, macrolides and lincosamides[47,48].

Opponents to the use of AGP also cite the example of Avoparcin. Vancomycin-resistant enterococci have not been isolated from healthy individuals and farm animals in the United States[49,50]. The primary source of VRE in the United States is hospital acquired infections[42]. In contrast, 12% of healthy individuals in Europe have been found to test positive for VRE[51]. This is argued to mean that while the problem is severe in the United States, it threatens only a small percentage of the population as compared to Europe[51].

Similar versions of this argument are ongoing throughout the scientific community, and due to the lack of peer reviewed literature and well planned and executed studies it is unlikely to be settled in the near future[41,26]. It is not the intent of this dissertation to argue the merits or risks of AGP usage, but rather to discuss the impact that alternatives might have in the event a poultry producer elects to use them or in the event they are removed from use in food animals by the FDA.

Future of AGP use in Poultry Production

The current rate of AGP use in animal agriculture is unlikely to continue in the future. Scientific and public scrutiny of the administration of therapeutic and sub-therapeutic doses of antimicrobials to animals has increased consistently since the release of the Swann report in the United Kingdom in 1969[52]. This report recommended that antibiotics used to treat infections in humans not be used as animal-feed additives[53]. As time has passed the level of scrutiny has increased. In 1986, Sweden was the first country to ban the use of AGP in animal agriculture[47,54]. Denmark followed suit and restricted the use of various antibiotics and AGP until a full ban of all AGP was enacted in 2000[55]. These bans were a result of data that suggested the use of AGP could increase the instance of resistance genes found in the microbial population and as a result pose a threat to human health[56,57]. In response to this potential threat, the World Health Organization and the Economic and Social Commission of the European Union (EU) publically stated that the use of antimicrobials in animals intended for food was a public health concern[57]. As a result of this proclamation, the EU formally put forth a plan to eliminate all AGP use in animal agriculture by January 1, 2006 in each member state[58]. Since that time only South Korea has formally restricted the use of all AGP in 2011[59]. The world's largest poultry producer, the United States, has made only limited efforts to restrict the use of antimicrobials and AGP. To date the only major action taken with regards to antibiotic use in animals was the complete prohibition in 2005 of fluoroquinolone use in animals due to the importance of floroquinolones in human health[56]. While having no formal ban in the United States, there has been a consumer driven demand to remove AGP from use. The social trend is driven by consumers and implemented primarily by large food service providers, such as McDonald's, Kentucky Fried Chicken, Wendy's, Hardees, Subway and Chipotle who are demanding meat providers to supply AGP free products[60]. This trend has been gaining momentum over the past decade and shows little sign of abating.

The scientific debate over the use of AGP is largely irrelevant to the negative public perception of AGP usage. As a result the poultry industry has been, and will continue, to be asked to limit the use of AGP and to seek alternatives.

Proposed Mechanism of Action of AGP

There is universal agreement from the scientific community that the mechanism of action of AGP is not well known. Conventionally, there have been four hypotheses put forth as to the mechanism of action: 1) AGP inhibit endemic subclinical infection, thus reducing the metabolic costs of the innate immune system; 2) they reduce microbial produced metabolites that inhibit growth rate (such as ammonia and bile degradation products); 3) they reduce microbial use of nutrients; and 4) they enhance the uptake and use of nutrients, because the intestinal wall in AGP-fed animals is thinner[61,55,62]. The underlying hypothesis is that the intestinal microflora is responsible for depression of animal growth and that the AGP through its control of the microflora is mitigating the negative effects.

An emerging hypothesis suggests reduced enteric inflammation is responsible for benefits associated with the addition of AGP[63]. Intestinal imbalances often are the result of changes, such as diet, infection, or even stresses that affect the intestinal microflora. All of these situations are commonly found in animal agriculture. When these imbalances occur, inflammation in the intestinal tract is increased and enteric bacterial populations are in a state of flux[64]. Niewold has argued that the effects of AGP on gut microflora may be due to effects on gut inflammatory status, rather than direct effects on the microflora[63]. Central to the hypothesis of Niewold, is that AGP may not benefit animals directly through an antimicrobial effect because they are provided at sub-minimal inhibitory concentrations, levels known to not inhibit affected pathogens within the ingesta of poultry. Additionally, the ability of antibiotics to affect growth rate and performance, regardless of the class of antibiotics used, and their target bacterial populations, suggests that the effects may not be directly due to antimicrobial activity. The microbial populations of the intestinal tract are extremely diverse, and as the animal ages research indicates gut associated microbial populations experience a great deal of change, becoming more and more complex[13]. It seems unlikely that a single AGP could exhibit a consistent positive growth response in such a situation. Niewold also pointed out that many popular AGP are classes that accumulate in phagocytes with known attenuation of the innate inflammatory response. This hypothesis is consistent with the observation that intestinal walls of AGP-fed animals are thinner, which could be attributed to a reduced influx and accumulation of inflammatory cells[65]. Additionally, it has been show that AGP have little effect on the intestinal microbiota populations, especially in the cecum. Though differences in populations of the ileum between AGP-fed and antibiotic free birds were noted, this may have been affected by other diet differences and could be attributed to the rapidly changing microbial population dynamics of the GIT[66]. The hypothesis put forth by Niewold could potentially explain the inconsistencies found by researchers investigating the loss of efficacy of some AGP over time. It can also be noted that there is a positive correlation with antibiotics that are used as AGP and anti-inflammatory capability[63]. Therefore, it may be possible that AGP act on the host, rather than the intestinal microflora.

Alternatives to AGP

As more sophisticated assays are developed, the scientific community will gain a better understanding of AGP. It has been argued that until we have a more full understanding of the true mechanism(s) of action our efforts to find alternatives to AGP will be handicapped[63]. However, as restrictions to AGP have become more prevalent, producers have turned to several technologies as alternatives to increase the efficiency of rearing food animals. These technologies include exogenous enzymes, organic acids, probiotics (direct-fed microbials), prebiotics, and herbal extracts or essential oils. These additives are intended to address a number of areas impacted by AGP[64]. This dissertation will focus primarily on probiotics as an alternative to AGP use; other alternatives will be only briefly discussed.

Exogenous enzymes

Growth promoting exogenous enzymes are primarily intended to act on non-starch polysaccharides (NSPs) which have been shown to have a negative impact on animal performance when included in the diet at high levels[67]. Non-starch polysaccharides, often referred to as dietary fiber in human nutrition, are a complex family of chemical structures that are found in the indigestible portion of food derived primarily from plants. These compounds include celluloses, pectins, oligosaccharides, arabinoxylans, and beta glucan[64]. Non-starch polysaccharides in animal diets are derived most commonly from the cereal components of the ration. Cereals vary in what NSPs are present, and the solubility of each NSP varies. Research indicates that solubility is correlated to the negative impact an NSP has on performance, higher solubility increases the negative impact[68]. Non-starch polysaccharides exert an anti-nutritive effect in poultry due to their viscous nature[68]. Soluble NSPs increase the bulk and viscosity of the ingesta, which decreases the rate of diffusion of both nutrients and enzymes, limiting the interaction of ingesta with the mucosal surface of the gut[68]. As a result, retention time of ingesta is prolonged in the small intestine and the rate of microbial fermentation is increased, placing the gut microbiome in direct competition with the animal for nutrients[68]. The rates at which these effects occur vary depending on the content, solubility, and family of NSP in the diet. Incorporation of AGP into NSP-containing diets have shown to mitigate the problem[69]. Inclusion of exogenous enzymes, such as xylanase in wheat based diets and beta-glucanase in barley based diets, has been shown to markedly reduce the negative impacts of diets high in NSPs[67,68].

Organic Acids

Organic acids (OA) are compounds that primarily include saturated straight-chain monocarboxylic acids and their respective derivatives, and are often referred to as fatty acids, volatile fatty acids, or weak or carboxylic acids[70,71]. Some of the most commonly used OA in food and feed additives are: propionic, acetic, citric, lactic, tannic, and butyric. They possess many properties that make them good candidates for alternatives to AGP, including antimicrobial and anti-inflammatory properties[72]. They also exhibit a large number of gut associated host effects[64,72,73]. Organic acids are weak acids by nature and pH is directly correlated with their ability to kill microbes because of the effect on concentration of undissociated acid[71]. Undissociated forms of OA can easily pass through the lipid membrane layer of bacterial cell walls and once internalized into the neutral pH of the protoplasm, they dissociate into anions and protons[74]. The increased proton concentration in the protoplasm requires the cell to expend energy through the ATP-driven proton pump to maintain a specific internal pH[71]. The end result can be impairment of cell function, death via energy depletion, and/or lysis[71]. Potential targets of OA include the cell wall, cytoplasmic membrane, and specific metabolic functions in the cytoplasm associated with replication, protein synthesis, and nutrient transport functions[75,71]. The antimicrobial activity of OA are influenced mainly by the following variables: (1) chemical formula, (2) pKa value of the acid, (3) chemical form (esterified or not, acid, salt, coated or not), (4) molecular weight, (5) the acid specific minimum inhibitory concentration (MIC) for the target microorganism, (6) the nature of the microorganism, (7) animal species, and (8) buffering capacity of the diet[76],[77]. Each acid has its own spectrum of microbial activity related to these factors. Also, additive effects of acids are possible. There are indications that the medium chain fatty acids may improve the efficacy of short chain fatty acids in controlling microbial populations[64]. In the field, mixtures of OA are mainly used, which makes their spectrum broader and combines the good qualities of different acids[78,73].

Physical form of the acids also plays a role in the AGP-replacement effect. The coating or micro-encapsulation of fatty acids with a progressive ‘slow release’ matrix is essential for their antimicrobial activity throughout the distal part of the GIT due to the fact that many organic acids can be rapidly absorbed and used directly as energy sources[64,73,79]. In addition to the antimicrobial properties of organic acids, their ability to act as a direct source of energy to gastrointestinal mucosa has been proposed as a potential explanation for the positive growth effects in livestock[64,73]. It has been well documented that OA exert a wide variety of effects on intestinal function in animals[64,72,73]. These include increased rates of mucosal development, and stimulation of epithelial cell proliferation and differentiation[80]. Additionally, OA also have documented anti-inflammatory effects and are central to maintaining intestinal integrity[81,64,73]. Thus for some acids, not only antibacterial, but also host effects can play a role in the AGP-replacement effect.

Probiotics

The beneficial effects of bacteria have been observed extensively throughout human history. Modern scientific investigation of beneficial bacteria are founded in the works of Nobel prize winner Eli Metchnikoff, who promoted the idea that yogurt and the bacteria it contained contributed to the longevity of Bulgarian peasants[82]. Over time these beneficial bacterial cultures have been referred to using different terms including, competitive exclusion cultures, probiotics and direct-fed microbials[83]. Most recently the term probiotic has become ascendant. The term “direct-fed microbial” is commonly differentiated as referring to beneficial live microorganisms that are consumed in the feed of animals intended for food production and is often used synonymously with probiotic. Probiotics can be defined as live microorganisms which when administered in adequate amounts confer a health benefit on the host[84]. The beneficial effects may include the reduction or exclusion of pathogenic bacteria, and has been previously referred to as competitive exclusion (CE) by Jaeger in 1974[85]. The term CE has also been adopted to describe a similar phenomenon first described by Nurmi and Rantala in 1973, where the ability of *Salmonella* to colonize the GIT of young chicks was greatly reduced by administration of a suspension of fecal material from healthy adult chickens[86,17]. These CE cultures are a subset of probiotics, and have been extensively researched. The benefits of probiotics are myriad and include the ability to decrease specific bacterial pathogens, decrease carcass contamination, increase body weight, increase the integrity of the GIT, decrease ammonia and urea excretion, reduce inflammatory reactions, improve mineral absorption, and increase immune function[87,8,88,89,90]. These characteristics place probiotics in the lead as a potential replacement for AGP in poultry.

The villus height to crypt depth ratio is thought to be indicative of intestinal health. Higher ratios indicate a healthier gut due to longer villus length, which is directly linked to surface area and absorption, while shorter crypt depth is indicative of a reduction of villus turnover. Awad *et al* noted that broilers given a *Lactobacillus* probiotic had significantly higher villus height to crypt depth ratios than control birds[91]. Tuomola *et al* demonstrated that some probiotic strains of *Lactobacillus* reduced the adhesion of pathogenic *E. coli* and *S.* Typhimurium to intestinal mucus while others increased mucus binding[92]. These findings indicate that not all probiotic strains have similar effects. In cell culture, *Bifidobacterium lactis* 420 supernatant was able to increase tight junction integrity and prophylactically protect tight junctions from damage by *E. coli* O157:H7[93]. Farnell *et al* studied the *in vitro* effects of multiple probiotic isolates on oxidative burst and degranulation[94]. The three isolates exhibiting the greatest effects *in vitro* were administered individually to day old chicks and heterophils were isolated for measurement of oxidative burst and degranulation 24 h later. All three treatment groups showed significant increases in the measured parameters as compared to untreated controls. Heterophils are important in controlling bacterial pathogens; as such the noted stimulation of heterophils may be one mechanism by which probiotics are able to reduce bacterial pathogens within the gut. Metabolites secreted by some strains of *Lactobacillus* have been shown to have anti-inflammatory effects[95,96,97]. When these metabolites were introduced into cell culture they caused a suppression of tumor necrosis factor alpha which could lead to a decrease of inflammation in the gut[96,97]. Menard *et al* reported further that this metabolite is able to cross the epithelial barrier and may be able to affect cells outside the GIT[96]. Further, transcriptional profiling of chickens fed probiotics suggested probiotic-induced differential regulation of multiple genes affecting innate immunity and apoptosis in the cecae of chickens, which may be a mechanism by which probiotics affect intracellular pathogens such as *Salmonella*[98].

Probiotics have been shown to improve the production parameters of commercial poultry. Vicente *et al* conducted a study in commercially housed broilers in Mexico to determine what, if any, contribution a commercially available probiotic culture would have[99]. The probiotic treated birds had a 0.9% reduction in mortality, a 2.06% improvement in body weight, and a 3.5% improvement in feed conversion as compared to non-treated controls[99]. Torres-Rodriguez *et al* evaluated the same probiotic in a similar trial in commercially housed turkeys in the United States[100]. An increase in body weight of 190 g and average daily gain of 1.63 g was observed in treated groups when compared to untreated controls. When costs were compared between treated and untreated groups, the cost per kilogram of meat was reduced by $0.0153 in the treated group[100]. Wolfenden *et al* observed a body weight increase of 8.7% over non-medicated controls and virtually identical increase as AGP treated birds in a trial conducted in commercially raised turkeys evaluating *Bacillus* spore based probiotic cultures[101]. It was also observed that birds receiving the probiotic treatment were significantly less likely than non-medicated controls to be infected with *Salmonella*, with a rate of recovery of 18% and 48% respectively[101]. No differences were observed in the AGP treated group. In addition to lower incidence of *Salmonella*, it was also noted that infected turkeys in the probiotic treated group had a significantly lower concentration of *Salmonella* in the ceca as compared to non-medicated controls[101].

Available scientific evidence suggests that probiotics may offer an effective alternative to AGP usage. It is often argued that probiotics do not consistently show performance benefits, and as such are not a reliable alternative. It is important to note that although AGP improve performance approximately 70% of the time in production animals, no measurable positive effects occur in almost one-third of applications[102]. Despite this observed rate of failure, AGP are used in abundance. Torres *et al* reported a similar success rate with a lactic acid bacteria-based probiotic in commercial turkeys[103]. The study utilized a total of 118 commercial turkey lots and the probiotic as administered to 60 flocks[103]. The weights of flocks from farms that historically ranked in the bottom 75% by the integrator were significantly increased ($P \leq 0.05$), whereas the weights of the flocks sold from the top 25% of farms were not significantly changed ($P \geq 0.05$)[103]. These data indicate for both AGP and effective probiotics, little positive effect would be anticipated in the best-performing flocks, possibly because these flocks were performing near maximum potential[102,103].

Prebiotics

Prebiotic was defined in 1995 by Gibson and Roberfroid as a non-digestible food ingredient which beneficially affects the host by selectively stimulating the growth of and/or activating the metabolism of one or a limited number of health-promoting bacteria in the intestinal tract, thus improving the host's microbial balance[104]. Prebiotics are primarily oligosaccharides based on hexose monosaccharides, including glucose, fructose, galactose, and mannose with a polymerization degree of between 2 and 20 monosaccharides[83]. Gibson and Roberfroid offered several criteria for a food ingredient to qualify as a prebiotic: it had to 1) be neither hydrolyzed nor absorbed in the upper part of the GIT; 2) be a selective substance for one or a limited number of beneficial bacteria commensal to the colon, which are stimulated to grow, are metabolically activated, or both; 3) be able to alter the colonic flora in favor of a healthier composition; and 4) induce luminal or systemic effects that are beneficial to the host health.[104] This definition is rather restrictive and often ingredients are referred to as prebiotics without fulfilling all four criteria[83,105]. A 2007 FAO report listed 400 commonly offered and used prebiotics, such as inulin, fructooligosaccharides, galactooligosaccharides, soy-oligosaccharides, xylooilgosaccharides, pyrodextrins, isomaltooligosaccharides, and lactulose[105]. The new, emerging prebiotic compounds listed included pecticoligosaccharides, lactosucrose, the sugar alcohols, glucooligosaccharides, levans, resistant starch, xylosaccharides, and soy-oligosaccharides, many were previously classified by Gibson and Roberfroid (1995) as not meeting the qualifications of a prebiotic[83].

The mechanism of action of prebiotics as an alternative to AGP is dependent on the nature of the compound, but their selective activity on beneficial bacterial populations in the GIT make them very similar to probiotics[104,64]. Prebiotic research in poultry has been limited when compared to human studies, and results are variable depending on the type of prebiotic examined[106]. Fructooligosaccharide (FOS) has been the dominant prebiotic studied for poultry production and has shown the ability to support growth of *Lactobacillus* and *Bifidobacterium*, while reducing *Escherichia coli* levels in ingesta[107]. FOS fed to broiler chickens at a concentration of 0.375% yielded consistent improvement in growth rate and feed efficiency[108]. Research indicates that inclusion of lactose in the diet of poultry can increase the efficacy of lactic acid bacterial based probiotics[109,110,100]. Poultry lack the ability to produce the enzyme to digest lactose, making it a prebiotic ingredient. Field trials conducted by Torres-Rodriguez *et al* have shown the inclusion of dietary lactose improved the effects of probiotics containing lactic acid bacteria, as was evidenced by 17.5% increased body weight of turkeys fed lactose in combination with a probiotic, compared to a 15.5% increase in turkeys fed only the probiotic, over untreated turkeys[100], thus justifying the combination of prebiotics and probiotics to improve performance.

Synbiotics

A combination of a prebiotic and a probiotic can be defined as a synbiotic[111]. The concept is that the combination could improve the survival and growth of the probiotic organism, because its specific substrate is available for fermentation, resulting in a more robust response[106]. There is also evidence that, in some cases, prebiotics impact growth negatively, and this can be dramatically reversed with the inclusion of a probiotic[109]. This observation could potentially explain inconsistent performance data available for prebiotics in poultry[109,100]. Research regarding poultry performance when using synbiotics is limited, but has more consistently shown positive results than prebiotics alone[109,110,100,112,113]. The previously mentioned research by Torres-Rodriguez and co-workers[100] exhibiting the increased performance observed with the lactose/lactic acid bacterial synbiotic application under commercial conditions exhibited body weight gains far greater than similar pen studies[109],[110]. Vicente *et al*[110] observed that the inclusion of a synbiotic in absence of enteric challenge resulted in no performance benefit, potentially explaining the superior performance observed by Torres-Rodriguez *et al*[100] under field conditions.

Plant Derived Products

Plants, plant extracts, and essential oils (EO) have long been used for food preservation and medicines[114]. These products have shown to exhibit many beneficial effects, such as enhancing the production of digestive secretions, stimulating blood circulation, exerting antioxidant properties, reducing the levels of pathogenic bacteria, and potentially enhancing the immune status[115]. Some of the bioactive antimicrobial chemical forms derived from plants include terpenoids, phenolics, glycosides, and alkaloids[64] Ginger, pepper, coriander, oregano, rosemary, sage, thyme, cloves, mustard, cinnamon, garlic, citrus, and tobacco are a few representatives of plant products expressing antibacterial properties[83]. Many of these same plant products are favored in homeopathic medicines. Essential oils have displayed inhibitory effects on a wide variety of bacterial species *in vitro*, including *Campylobacter jejuni*, *Clostridium sporogenes*, *Escherichia coli*, *Listeria monocytogenes*, *S. typhimurium*, and *S. pullorum*[116,117]. Mitsch *et al* tested the effects of two different blends of EO on *Clostridium perfringens* in broiler chickens[118]. The two blends contained varying concentrations of EO from thyme (thymol) and oregano (carvacrol), with consistent levels of clove (eugenol), turmeric (curcumin), black pepper (piperin), and were fed at 100 ppm throughout the study. The first blend reduced *C. prefringens* in the feces at day 14, 21, and 30; in the upper ileum and ceca on day 14 and 21; and in the cloaca on day 14[118]. The second blend reduced *C. perfringens* in the upper ileum on day 14 and 30 and in the cloaca on day 30[118]. Additionally, *Yucca schidigera* extract was shown to have a synergistic effect when incorporated in the diet of broilers vaccinated against coccidiosis[119]. Broilers receiving the extract demonstrated significant improvements to average daily gain and feed conversion, as well as more rapid early gut development. Studies testing a blend of oregano and yucca extracts, along with organic minerals, improved average body weights, feed conversion and mortality of broilers challenged with *H. meleagridis*[120]. Another study using the same blend supplemented in broiler diets, showed a reduction in the severity of intestinal lesions caused by *Eimeria tenella*, compared to untreated positive controls.

When considered all together, multiple options exist to provide similar benefits as those conveyed by AGP. In fact, when administered in combination, it may be possible to improve upon the health and performance gains delivered by AGP, thus having significant impact on welfare and profitability in the poultry industry.

Pre-Harvest Carcass Weight Loss (Shrinkage)

Transport of live animals also has important implications in both economic and welfare areas[121]. In poultry and other species, economic losses during transport are due to mortality, carcass shrinkage (carcass dehydration) and carcass condemnation[122]. Modern poultry producers have made efforts to minimize losses, but there are several factors that limit interventions. The primary contributing factor to loss in poultry is shrinkage, which is exacerbated by the practice of pre-harvest feed withdrawal (FW). Although FW contributes to shrinkage, USDA inspectors have a zero tolerance policy when it comes to visible ingesta on poultry carcasses in the processing plant[123]. To achieve this standard and minimize ingesta content in the gut of poultry, producers are required to cease feed intake prior to harvest[124]. Feed passage time of poultry dictates the time required to comply with the USDA food safety mandate[125,126]. Feed withdrawal prior to processing of poultry is the most commonly employed method to reduce ingesta contamination during processing[125,126]. However, shrinkage begins immediately after FW, resulting in recommendations that slaughter take place within six hours of onset to minimize the shrink-associated losses[127,122]. Thus, processing schedules are organized to consider FW effects on both gut fullness and shrinkage[126]. Recent trends have placed scrutiny on the impact that feed restriction in long term and forced FW has on meat type poultry[128,129,130,131]. Research to mitigate the problems associated with FW has yet to yield solutions the poultry industry has adopted[125]. Special diet formulations have shown success in improving weight retention during transport[132,133,134]. Nijdam *et al* provided diets during the last phase of life with alternative formulations to provide high energy content, different macronutrient composition, and low crude fiber content to reduce the negative effects of feed withdrawal and transport, without an increased content of the digestive tract[133]. Farhat *et al* and Rathgeber *et al* provided a highly digestible feed supplement during the on-farm FW hours in chickens and turkeys respectively[132,134]. While these strategies appear to have created significant benefits, to date they have not been adopted by the poultry industry.

Shrinkage is greatly compounded by the removal of water during FW[135]. To combat the additional shrinkage associated water restriction poultry producers maintain water availability for as long as possible. Jarquin *et al* observed that while poultry continue to consume water during FW, the rate is much lower than when feed is present[136]. In studies conducted by Wolfenden *et* *al* broiler chickens show a commercially available OA water treatment product significantly reduced carcass condemnation at the processing plant and mortality during transportation, with consistent improvement of average body weights at the farm and at the processing plant[137]. This study is the first of its kind that exhibited an OA product had the ability to increase water consumption. In the present study, this product was used in commercial turkey production to evaluate shrinkage during FW as well as during the transportation to the processing plant.

Summary

Considering the projected demands for poultry meat and the challenges associated with increasing production while maintaining market viability, the poultry industry will be forced to search for ways to improve upon existing standards. The potential loss of one of the main tools currently being used for performance enhancement, AGP, will require the poultry industry to make use of existing alternatives, apply existing technologies in different ways, or develop more effective alternatives. Published reports indicate there are a variety of viable alternatives to AGPs. The use of probiotics to increase growth rate, reduce disease, and control specific food borne pathogens has been well documented in a variety of experimental and commercially available formulations, suggesting they could offer a quick solution to the dilemma. To date, the easiest opportunity to improve cost of goods sold has been to focus on the single largest expense associated with raising poultry, feed, which is reported to contribute 65% of the total cost[138]. As grain prices rise and the competition for feed ingredients becomes more intense it is unlikely the impact of feed efficiency will diminish. However, other opportunities to cut costs or increase value exist. This can be seen in products such as antibiotic free, organic, or free range poultry, which sell for a premium price. Exploiting areas of known losses will become necessary as well, even when the profit contribution is small. Improvements in animal health and welfare will be paramount in the effort to maintain profitability, due to their impacts on efficiencies and costumer acceptance. The ability to supply a product the customer is willing to purchase is a must. Research presented in the following chapters evaluated commercially available products for their impact on production parameters and potential to replace AGP in commercial poultry.

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Chapter III

EVALUATION OF DIRECT-FED MICROBIALS AS ALTERNATIVES TO ANTIBIOTIC GROWTH PROMOTERS IN MODERN POULTRY PRODUCTION

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Key words: Probiotics, DFM, *Bacillus*, Broiler, Performance, Alternative to AGP, Antibiotic Free, AGP Free

Running Title: DFM as alternatives to AGP

Primary Audience: Researchers, Nutritionists, Feed Manufacturers, Producers, Veterinarians

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III. EVALUATION OF DIRECT-FED MICROBIALS AS ALTERNATIVES TO ANTIBIOTIC GROWTH PROMOTERS IN MODERN POULTRY PRODUCTION

Summary

In a series of experiments the suitability of replacing antibiotic growth promoters (AGP) with direct-fed microbials (DFM) was evaluated. In experiments 1 and 2, broiler chickens were kept in floor pens on used litter and grown to market age. Experiment 1 compared a standard diet containing no AGP or DFM, standard diet plus 50 grams per ton of bacitracin methylene disalicylate (BMD), or a standard diet including Sporulin® - a commercially available *Bacillus subtilis* spore-based DFM. Experiment 2 compared standard diets containing either 50 grams per ton of BMD or Sporulin. Experiment 3 was conducted in commercial broiler houses comparing the integrator's AGP free diet with a diet containing Sporulin. The data from these three experiments indicate that inclusion of a *Bacillus* DFM can improve performance as compared to an AGP free diet or one containing traditional AGP. This suggests that as AGP availability becomes limited, poultry producers may look to DFM as an alternative to maintain efficient production.

Description of Problem

Recent trends in the animal agriculture industry have been pushing for production methods which are perceived to be more natural and sustainable. These trends are primarily shaped by social pressures from consumers in the general public. As a result, the industry is looking for ways to reduce or eliminate the inclusion of chemotherapeutic compounds with a negative public perception. Antibiotic growth promoters (AGP) in particular have been singled out due to the emergence of antibiotic resistant bacteria over the years[1] and the prevalence of press regarding these organisms in popular media. While the major driving force behind the movement is consumer demand, in some cases, government organizations have enacted bans on inclusion of AGP. In 2006, a full ban on the use of AGP was enacted in the European Union [2] and in 2011 South Korea followed suit[3]. While these two cases are significant, the overall driving force behind the movement has been consumer driven and the response of food providers to consumer trends. Many popular restaurant chain companies such as McDonald's, Kentucky Fried Chicken, Wendy's, Hardees, Subway and Chipotle have required suppliers to guarantee meat they purchase has been grown without the inclusion of AGP in feed[4]. The scientific debate over AGP is largely irrelevant to the negative public perception. As a result, the poultry industry has been, and will continue to be, incentivized to limit the use of AGP and to seek alternatives.

The exact percentage of companies currently utilizing AGPs in feed is not known. Published estimates from 2000 state that the majority of broiler feeds were incorporating some AGP, but usage rate was in decline[5]. Bacitracin methylene disalicylate (BMD) was the most abundantly used AGP. It is a branched, cyclic decapeptide compound that interferes with cell membrane function, suppresses cell wall formation by preventing the formation of peptidoglycan strands, and inhibits protein synthesis[6]. BMD is approved by the United States Food and Drug Administration for inclusion in broiler feeds for the purpose of increasing rate of weight gain, improving feed efficiency, and as an aid in the prevention of necrotic enteritis caused by or complicated by *Clostridium* spp. or other organisms susceptible to BMD[7]. Contemporary scientific documentation regarding the efficacy of BMD as an AGP is limited. A literature review of BMD use in broilers since 2000 indicates that in pen trials on used litter BMD provides an increase in growth rate of $\leq 1.7\%$ in male broilers and slightly better performance in females[8,9,10]. Previous review of the efficacy of AGP has reported varying levels of performance benefits over time, and it is interesting to note that in approximately one third of all applications there is no measurable performance benefit [11].

Sporulin, a commercially available *Bacillus subtilis* spore based DFM, has been marketed in the United States since 2010 as an option for poultry producers looking to replace AGP. It contains three strains of *B. subtilis* selected for their ability to propagate in the intestinal tract of poultry, improve growth rates, and compete with pathogenic bacteria[12,13,14]. In this study, experiments were conducted to assess the suitability of Sporulin as an alternative to commonly used AGPs and to evaluate the impact of including DFMs in AGP free production.

Materials and Methods

Experiment 1

Day of hatch male broiler chicks (Ross X Ross 708) were obtained from a commercial hatchery and allocated randomly in floor pens containing used litter at a density of 0.83 ft^2 per bird. All chicks received standard vaccinations in the hatchery. Chicks were allocated into groups of 20 with 10 replicates per treatment. Treatments consisted of 50 grams per ton of BMD, $1x10^6$ CFU/g of Sporulin included continuously in the diet, or a control diet containing no AGP or DFM. A commercial diet that met or exceeded NRC requirements and water were provided *ad libitum* through the duration of the experiment. The broilers were raised to 46 days of age and data was collected throughout the trial period.

Experiment 2

As in Experiment 1, day of hatch commercial cross male broiler chicks (Cobb) were obtained from a commercial hatchery and allocated randomly in floor pens containing used litter at a density of 0.83 ft^2 per bird. All chicks received standard vaccinations in the hatchery. Chicks were allocated into groups of 54 with 12 replicates per treatment. Treatments consisted of either 50 g per ton of BMD, or $1x10^6$ CFU/g of Sporulin included continuously in the diet. Feed and water were provided *ad libitum* through the duration of the experiment. The broilers were raised to 38 days of age and data was collected throughout the trial period.

Experiment 3

A third experiment was conducted on commercial cross broilers (Cobb) under field conditions comparing an industry standard antibiotic free program to a program modified to improve enteric health. The veterinarian-designed enteric health program included continuous treatments with an in-feed DFM (Sporulin) in combination with periodic treatment with a water administered probiotic (FloraMax®-B11) on days 1, 8 and 18 and a water acidifier (Optimizer™) on days 7 and 17. The treatment group, consisting of 7 flocks (225,000 birds), was compared to a control group of 12 flocks (400,000 birds). The experiment was conducted on a single farm location with multiple houses, where treatments were randomly assigned among houses. The broilers were raised to an average of 33 days of age and data was collected throughout the trial period.

Statistical Analysis

Differences in measured parameters between groups were determined by ANOVA using the GLM procedure. Significant differences ($P < 0.05$) were further separated using Duncan's multiple range test. All statistical analysis was conducted using the SAS v9.3 edition[15].

Results and Discussion

In Experiment 1 no significant differences were observed, however, numerical improvement was consistently observed in the group receiving Sporulin compared to other groups (Table1). At termination, BW was heaviest in the Sporulin group (3.53 kg) as compared to the Control group (3.48 kg) and BMD treated group (3.47 kg). Average daily gain was greatest in birds fed a diet containing Sporulin (76.7 g/d), followed by the control diet (75.7 g/d) and BMD treated diet (75.4 g/d). Similar trends were observed in FCR, birds treated with Sporulin (1.71) outperformed birds in the BMD (1.76) and Control (1.77) groups. The observed improvement in FCR, while not significant, had a P-value = 0.06 for Sporulin versus Control and P = 0.15 for Sporulin versus BMD. FCR was adjusted for mortality and to a standard body weight of 3.175 kg using a ratio of .01 points FCR: 23 g BW.

It was hypothesized that the low number of birds per group and the minimal number of replicates resulted in numeric but not significant differences in Experiment 1. Therefore, in Experiment 2, the design was altered in an attempt to more fully evaluate the hypothesis that Sporulin was an effective alternative to AGP. In this altered experimental design significant differences were observed in FCR and the previously observed trend of superior performance in broilers consuming Sporulin was confirmed. BW and ADG at termination were heavier in the Sporulin treated groups (2236 g: 57.6 g/day) versus those receiving BMD (2207 g: 56.8 g/day) with a P value of 0.07. FCR was significantly ($P \leq 0.05$) improved in the DFM treated groups (1.60) versus the BMD groups (1.68). FCR was adjusted to a standard body weight of 2.267 kg using a ratio of .01 points FCR: 27.2 g BW. A summary of the data can be found in Table 2.

Under commercial flock conditions in Experiment 3, it was observed that the treated flocks had significantly ($P \leq 0.05$) higher BW and ADG (1726 g: 49.61 g/day) versus the control flocks (1540 g: 45.22 g/day; Table 3). Improvement ($P < 0.05$) in FCR from 1.66 in treated flocks to 1.88 in control flocks was also observed (Table 3). Feed conversion ratio was adjusted to a standard body weight of 1.6 kg using a ratio of .01 points FCR: 27.2 g BW.

The beneficial effects of bacteria have been observed extensively throughout human history, most notably by Nobel prize winner Eli Metchnikoff, who promoted the idea that yogurt and the bacteria it contained contributed to the longevity of Bulgarian peasants[16]. Over time these beneficial bacterial cultures have been referred to using different terms including, competitive exclusion cultures, probiotics and direct fed microbials[17]. The term "direct-fed microbial" is commonly differentiated in animal agriculture as referring to beneficial live microorganisms that are consumed in the feed of animals intended for food production and is often used synonymously with probiotic, although technically this term was generated by an FDA definition in a compliance policy guide[18]. Probiotics can be defined as live microorganisms which when administered in adequate amounts confer a health benefit on the host[19]. The beneficial effects may include the reduction or exclusion of pathogenic bacteria, this is what was previously referred to as competitive exclusion (CE) by Jaeger in 1974[20]. The term CE has also been adopted to describe a similar phenomenon described by Nurmi and Rantala in 1973, where the ability of *Salmonella* to colonize the gastro-intestinal tract of young chicks was greatly reduced by the administration of a suspension of fecal material from healthy adult chickens[21,22]. These CE cultures are a subset of probiotics, and have been extensively researched. The benefits of probiotics in poultry are myriad and include the ability to decrease specific bacterial pathogens, decrease carcass contamination, increase body weight, increase the integrity of the gastrointestinal tract, decrease ammonia and urea excretion, reduce inflammatory reactions, improve mineral absorption, and increase immune function[23,24,25,26,27]. These characteristics place probiotics in the lead as a potential replacement for AGP in poultry.

Probiotics have been shown to improve the production parameters of commercial poultry, consistent with the data presented here. Vicente *et al* conducted a study in commercially housed broilers in Mexico to determine what if any contribution a commercial available probiotic culture would have[28]. The probiotic treated bird had a 0.9% reduction in mortality, a 2.06%

improvement in body weight, and a 3.5% improvement in feed conversion as compared to non-treated controls. Torres-Rodriguez *et al* evaluated the same probiotic in a similar trial in commercially housed turkeys in the United States[29]. An increase in body weight of 190 grams and average daily gain of 1.63 g was observed in treated groups when compared to untreated controls. When costs were compared between treated and untreated groups, the cost per kilogram of meat was reduced by $0.0153 in the treated group[29]. Wolfenden *et al* observed a body weight increase of 8.7% over non-medicated controls and virtually identical to AGP treated birds in a trial conducted in commercially raised turkeys evaluating *Bacillus* spore based probiotic cultures[14]. It was also observed that birds receiving the probiotic treatment were significantly less likely than non-medicated controls to be infected with *Salmonella* with a rate of recovery of 18% and 48% respectively[14]. No differences were observed in the AGP treated group. In addition to lower incidence of *Salmonella*, it was observed that infected turkeys in the probiotic treated group had a significantly lower concentration of *Salmonella* in the ceca as compared to non-medicated controls[14].

Available scientific evidence suggests that probiotics may offer an effective alternative to AGP usage. It is often argued that probiotics do not consistently show performance benefits, and as such are not a reliable alternative. It is important to note that although AGP improve performance approximately 70% of the time in production animals, no measurable positive effects occur in almost one-third of applications[11]. Despite this observed rate of failure, AGP are used in abundance. Torres *et al* reported a similar success rate with a lactic acid bacteria-based probiotic in commercial turkeys[30]. The study utilized a total of 118 commercial turkey lots and the probiotic was administered to 60 flocks[30]. The weights of the flocks from farms that historically ranked in the bottom 75% by the integrator were significantly increased ($P \leq 0.05$), whereas the weights of the flocks sold from the top 25% of farms were not significantly changed ($P \geq 0.05$)[30]. These data indicate for both AGP and effective probiotics, little positive effect would be anticipated in the best-performing flocks[11,30].

Data from these pen trials and a large commercial field trial clearly indicated that Sporulin was a suitable candidate as a replacement to AGP, showing favorable results when compared to the most commonly used AGP, BMD, in conventional poultry production. Sporulin consistently increased performance over BMD in all experiments, increasing BW by greater than 1.5% and an improvement in FCR of greater than 2.3%. Sporulin inclusion also showed strong performance benefits when incorporated into AGP free production. Growth rates of broiler chickens under existing industry conditions increased dramatically. BW increased by 9.7% and FCR improved by 7.8%. Feed costs contribute 65% of the total price of rearing poultry, amplifying the significance of improved FCR[31]. When poultry producers achieve improvements in FCR, the cost of goods is directly lowered, and even modest changes can account for significant profitability adjustments. Probiotics are well within the category of socially accepted performance enhancers, and seem to offer a strong solution to the pending quandary on how to replace AGP.

Conclusions and Applications

1. Inclusion of Sporulin in the diet significantly improved BW, FCR and ADG.
2. This specific *Bacillus* DFM may provide a viable alternative to the use of AGP in broilers.

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Tables

Table 1: Experiment 1 - Evaluation of Sporulin® as an alternative to BMD as an AGP

Treatment	BW (kg)	ADG (g)	FCR[1]
Standard Diet (No AGP, No DFM)	$3.48^{a}\pm0.03$	$75.7^{a}\pm0.74$	$1.77^{a}\pm0.02$
Standard Diet + 50 g per ton BMD[2]	$3.47^{a}\pm0.03$	$75.4^{a}\pm0.73$	$1.76^{a}\pm0.03$
Standard Diet + Sporulin[3]	$3.53^{a}\pm0.03$	$76.7^{a}\pm0.68$	$1.71^{a}\pm0.02$[4]

[1] Adjusted for mortality and to a standard body weight of 3.175 kg using a ratio of .01 points FCR: 23 g BW

[2] Bacitracin methylene disalicylate

[3] Pacific Vet Group-USA, Inc.

[4] P = 0.06 vs. Standard Diet; P = 0.15 vs Standard Diet + BMD

[a,b] denote significant statistical difference ($P \leq 0.05$)

n = 10 pens per treatment, 20 birds per pen

Table 2: Experiment 2 - Evaluation of Sporulin® as an alternative to BMD as an AGP

Treatment	BW (kg)	ADG (g)	FCR[1]
Standard Diet + 50 g per ton BMD[2]	2.20[a]±0.007	56.8[a]±0.19	1.68[a]±0.005
Standard Diet + Sporulin[3]	2.23[a]±0.013[4]	57.6[a]±0.36[4]	1.60[b]±0.006

[1] Adjusted for mortality and to a standard body weight of 2.267 kg using a ratio of .01 points FCR: 27.2 g BW

[2] Bacitracin methylene disalicylate

[3] Pacific Vet Group-USA, Inc.

[4] $P = 0.07$ vs. Standard Diet + BMD

[a,b] denote significant statistical difference ($P \leq 0.05$)

n = 12 pens per treatment, 54 birds per pen

Table 3: Experiment 3 - Evaluation of probiotics as an non-antibiotic performance enhancer as part of an enteric health program in commercial broilers

Treatment	Age (d)	BW (kg)	ADG (g)	FCR[1]
Standard Diet (No AGP, No DFM)	32.8[a]±0.92	1.54[b]±0.034	47.1[b]±0.001	1.88[a]±0.05
Standard Diet + probiotics[2]	33.7[a]±1.55	1.69[a]±0.047	51.2[a]±0.001	1.66[b]±0.04

[1] Adjusted to a standard body weight of 1.6 kg using a ratio of .01 points FCR: 27.2 g BW

[2]Sporulin® included in all feed; FloraMax®-B11 treatment in water on day 1, 8, and 18; Optimizer™ treatment on day 7 and 17 – all used according to label requirements

[a,b] denote significant statistical difference ($P \leq 0.05$)

Standard diet n = 12 flocks, 400,000 birds total

Standard diet + probiotics n = 7 flocks, 225,000 birds total

Appendix

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To Whom It May Concern,

The first author of enclosed paper, "Evaluation of Direct-Fed Microbials as Alternatives to Antibiotic Growth Promoters in Modern Poultry Production", is Christopher M. Pixley. Mr. Pixley was primarily responsible for the work and research associated with this paper, and completed greater than 51% of the work.

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B.M. Hargis

Chapter IV

EVALUATION OF A COMMERCIALLY AVAILABLE ORGANIC ACID PRODUCT DURING FEED WITHDRAWAL AND ITS RELATION TO CARCASS SHRINK IN COMMERCIAL TURKEYS

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International Journal of Poultry Science 9 (6): 508-510, 2010

ISSN 1682-8356

IV. EVALUATION OF A COMMERCIALLY AVAILABLE ORGANIC ACID PRODUCT DURING FEED WITHDRAWAL AND ITS RELATION TO CARCASS SHRINK IN COMMERCIAL TURKEYS

Abstract

The transport of live animals has important economic and welfare implications. A commercially available organic acid product (Optimizer™) was added to the drinking water of commercial hen turkeys during pre-slaughter feed withdrawal (FW) in two trials. In trial 1, a total of 60 trailers from treated (OA) or control non-treated turkey houses were evaluated. Turkey farmers initiated water treatment on the day before pick up (8-12 h treatment according to label directions). Investigators recorded trailer numbers as they were loaded out of each house to confirm which trailers contained treated birds vs. control non-treated birds. Individual trailer weights were recorded upon arrival to the processing plant and again immediately prior to live hang. A significant reduction in rate of weight loss during holding at the processing plant was observed in the treated turkeys (719 g/min per OA treated trailer vs. 845 g/min per control trailer). In trial 2, two commercial market age turkey houses were selected and in each house, 400 birds were weighed and recorded as a representative sampling. The treated house received OA administered according to manufacturer's directions continuously for 19 h. At the end of this time, 400 birds were weighed and recorded as a representative sampling. A significant ($p<0.05$) improvement of average body weights was observed in treated turkeys during 19 h (125 g treated vs. 35 g control), an average of 90 grams difference. Experiments are ongoing to measure water consumption during the FW that may explain the reduction in carcass shrinkage during transportation to the processing plant and increased body weights at the farm by increasing hydration of turkeys treated with OA.

Introduction

The transport of live animals has important implications in both economic and welfare areas[1]. In poultry and other species, economic losses during transport are due to mortality, carcass shrinkage (carcass dehydration) and carcass condemnation[2]. Although pre-transport feed withdrawal (FW) contributes to carcass shrinkage, FW prior to processing of poultry is simple and is commonly employed to reduce ingesta contamination during processing[3,4,5]. However, shrinkage begins immediately after FW[2,6,7], resulting in recommendations that slaughter take place within 4-6 h after FW to minimize the shrink-associated losses. Thus, processing schedules should consider FW effects on both gut fullness and shrinkage. Previously our laboratory conducted a study in broiler chickens showing a commercially available water treatment product significantly reduced carcass condemnation at the processing plant and mortality during transportation, with consistent improvement of average body weights at the farm and at the processing plant[8]. In the present study, this product was used to evaluate shrinkage during FW as well as during the transportation to the processing plant.

Materials and Methods

Organic acids: A commercially available water treatment product (Optimizer™) was used in the drinking water according to manufacturer's directions. This commercial product is a proprietary combination of organic acids and flavoring agents. Previous publications have also shown this product, under experimental conditions, to reduce Salmonella colonization in crop and cecal tonsils without affecting water consumption in chickens[9,10].

Trial 1

Trial 1 was conducted with a total of 60 trailers from treated and control non-treated turkey houses. Each trailer carried an average of 2100 market age turkeys from a commercial-cross turkey line. These turkeys were being raised by contract farmers for an integrated turkey company in the state of Arkansas, USA. Water treatment was initiated at 9 PM on the day before pick up with sufficient OA stock solution to last 8-12 h, during the time of FW (time off feed). Investigators recorded trailer numbers as they were loaded out of each house to confirm which birds received the OA vs. controls. Individual trailer weights were recorded upon arrival at the processing plant (Time 1) and immediately prior to live hang (Time 2).

Formulas used

Yard Time = Time 2 - Time 1

Shrink = Trailer weight at Time 1 – Trailer weight at Time 2

Shrink/minute = Shrink/Yard Time

% Shrink/minute = ((Shrink/Trailer weight at Time1)/Yard Time in minutes)100

Time off feed = Time 2 - Time when feed access was removed

Value of treatment = (Control Shrink-Treatment Shrink) (Value of the carcass per Kg)

Benefit to Cost Ratio = Value of treatment/OA product cost

Trial 2

In trial 2, two commercial market age turkey houses were selected and a representative sample (n = 400) was weighed and recorded. Portable fencing was used to corral approximately 20 turkeys at approximately 20 sites for weighing. The treated house received the mix of OA continuously for 19 h. At the end of this time, a representative sample (n = 400) was weighed and recorded.

Data analysis

In trial 1, data collected were subjected to one-way analysis of variance for carcass shrinkage during holding at the processing plant yard prior to live hang and significant differences between means were further separated using Duncan's multiple range test[11]. In trial 2, a two by two factorial analysis was performed to evaluate body weights before and after treatment in the OA treated vs. control non-treated turkeys. Statistical significance was designated at $p<0.05$ in both trials.

Results and Discussion

Economic losses during transport are due to mortality, particularly of pigs and poultry, carcass bruising and shrinkage (loss of weight) and reductions in meat quality[12]. Table 1 shows the effect of this OA product administered during turkey FW on carcass shrinkage during holding at the processing plant in trial 1. A significant reduction in carcass shrinkage in the turkeys that received the mix of OA was observed when compared with the control non-treated birds. There were no significant differences in the time off feed in the FW period or the transit time of the trailers from the farms to the processing plant between the treated and the control non-treated birds. Table 2 summarizes the effect of OA during feed withdrawal on body weights of commercial turkeys before and after the treatment in trial 2. A significant increase in the body weight of the treated turkeys that received OA was observed when compared with the non-treated turkeys, with 90 grams gained in only 19 h of treatment with OA. Economic estimates in commercial broiler chickens that received a similar treatment of OA during FW suggested a tenfold return on investment after deducting the cost of the OA product[8]. In the present study, the benefit to cost ratio was estimated at greater than 6.5:1. Note that weight loss during transportation from farm to the processing plant was not measured. The losses were only quantified during the time the trailers spent in queue at the processing plant.

In areas where there are regulatory and consumer issues with *Salmonella* contamination of carcasses, there may be an additional advantage to some OA products. This product has shown to decrease *Salmonella* in market age broilers when administered during the pre-slaughter FW period[9]. Previous research has suggested that administration of lactic acid during the pre-slaughter FW, effective for reducing crop contamination with *Salmonella* at relatively high concentrations, could discourage water consumption and lead to excessive carcass shrinkage[13]. While this evidence was shown when using lactic acid alone, the product evaluated in the present study is reported to contain a proprietary combination of organic acids and flavorants where water consumption is not discouraged. Flavoring agents claimed by the manufacturer have not been released or evaluated. Organic acids are a readily available energy source for both the birds and gut microflora; therefore, it is important that the organic acids be administered in sufficient concentrations to be bactericidal, but low enough concentrations to be voluntarily consumed by the birds.

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Tables

Table 1: Evaluation of the effect of organic acids during feed withdrawal on carcass shrink during holding of commercial turkeys at the processing plant in trial 1

	Control	Treated
Yard Shrink per trailer per minute (grams)	845^{a}±27.1	719^{b}±22.6
Shrink per minute (%)	2.8^{a}±0.045	2.4^{b}±0.044
Transit time (min)	71^{a}±5.96	68^{a}±6.08
Time off feed (min)	809.14^{a}±40.1	726.33^{a}±30.9
Difference of shrink per minute between groups	126 grams	

Values are presented as mean ± SE. Different letters within rows of experimental columns indicate significant differences between treatments (P<0.05).

Table 2: Evaluation of organic acids during feed withdrawal on body weights on commercial turkeys before and after treatment in trial 2

	Body weights before treatment (0 h)	Body weights after treatment (19 h)	Difference in body weights
Control	$6359^{b,x}$±40	$6394^{b,y}$±35	35 grams
Treated	$6456^{b,x}$±36	$6581^{a,x}$±33	125 grams
Difference in body weights	97 grams	187 grams	90 grams

Values are presented as mean ± SE. Different letters within rows of experimental columns indicate significant differences between treatments (P<0.05). Different letters within rows (a,b) or within columns (x,y) indicate significant differences

Appendix

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To Whom It May Concern,

The first author of enclosed paper, "Evaluation of a Commercially Available Organic Acid Product During Feed Withdrawal and its Relation to Carcass Shrink in Commercial Turkeys", is Christopher M. Pixley. Mr. Pixley was primarily responsible for the work and research associated with this paper, and completed greater than 51% of the work.

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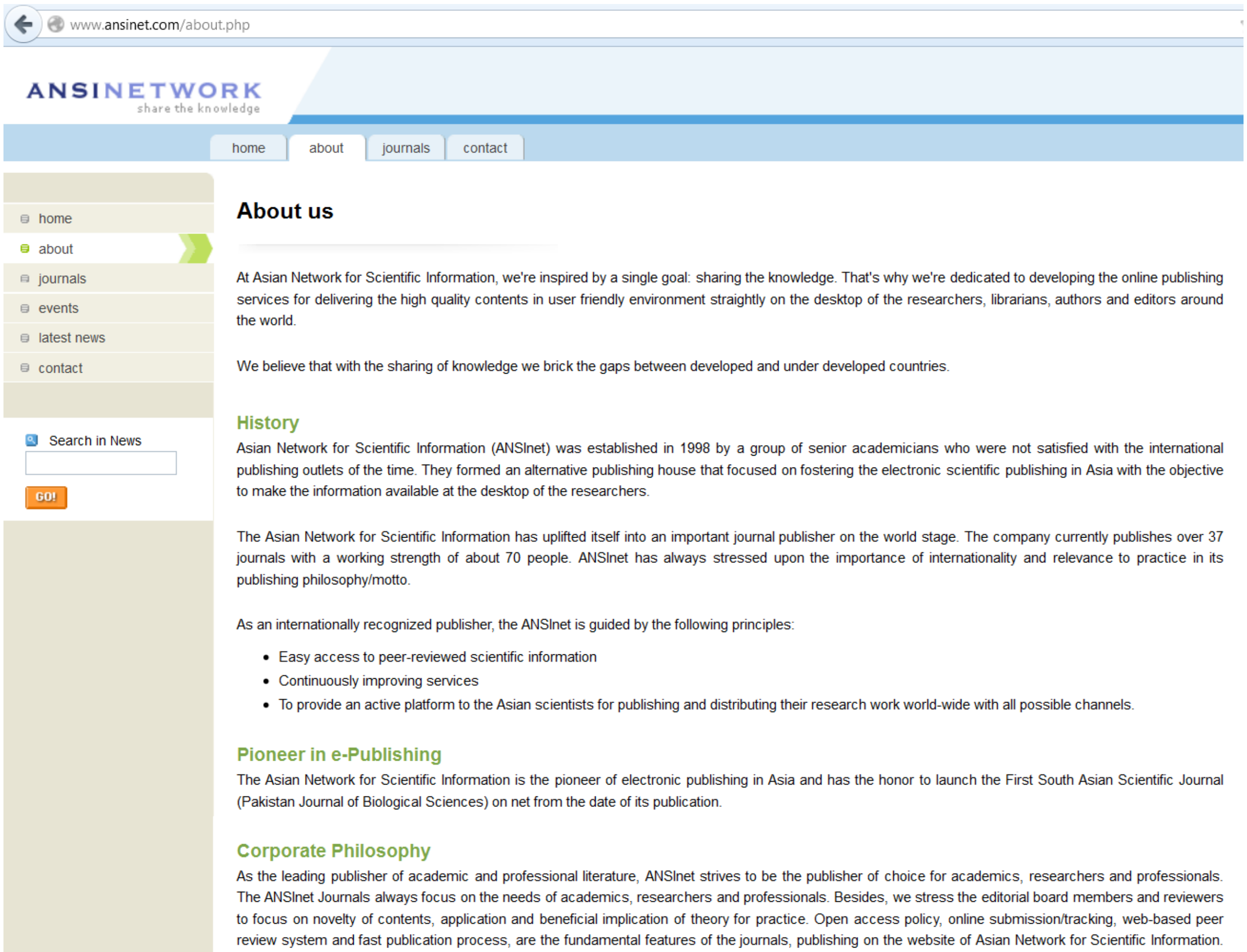
B.M. Hargis



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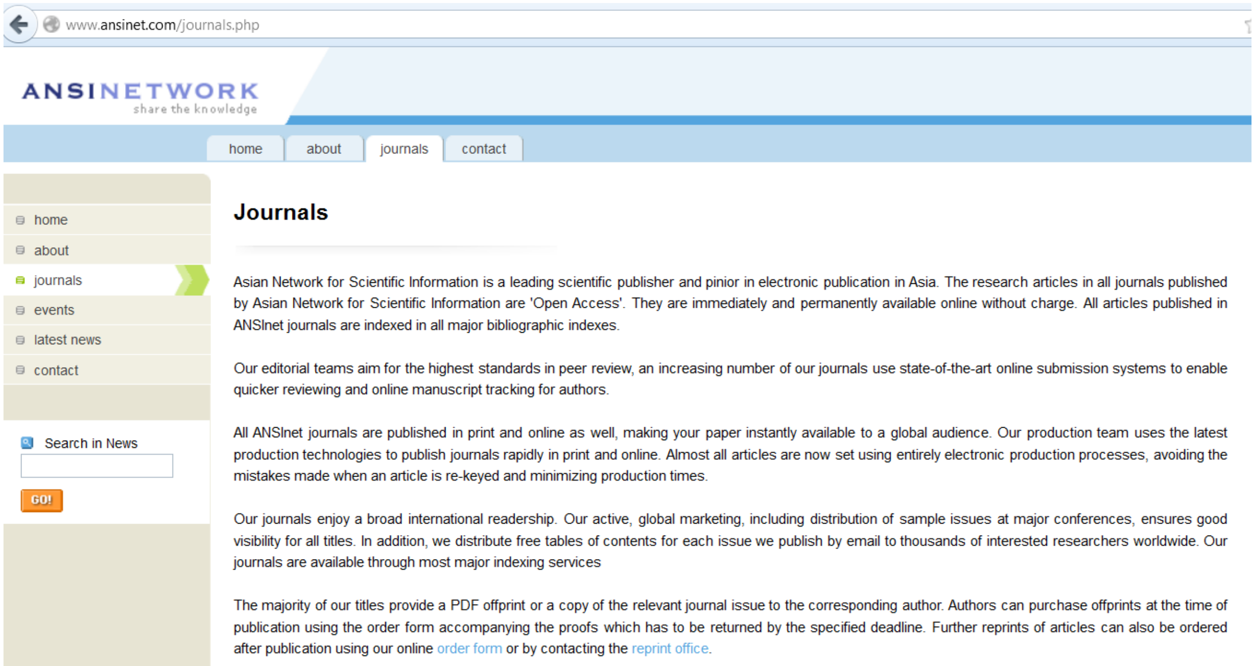

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V. Conclusions

Historically, AGP have been added to poultry feeds because of their proven benefits on growth parameters[1]. Recent consumer trends, largely based on controversial arguments that AGP pose a risk to human health due to increased antibiotic resistance in pathogens[2], have begun demanding that food animals be raised without the aid of AGP. Additionally, AGP have already been removed as an option for production by government agencies in South Korea[3] and Europe[4], and producers are increasingly concerned with the possibility of the same occurrence in the US, which has spurred an increasing interest in research aimed to provide viable alternatives for AGP. However, the mechanism of AGP action(s) is not well known[5], which has led to multiple areas of focus in the search for replacement technologies[6], and it is at least somewhat likely that combinations of such emerging technologies will be needed to realize the same benefits conferred by AGP. The objective of the enclosed body of work was to establish what, if any, impact the use of specific non-traditional technologies might have on modern poultry production. These technologies would need to meet the criteria of a discerning market that is focused on removal of chemotherapy and improving the welfare of animals used in the animal agriculture business. In order to be widely accepted, these technologies would also need to improve the efficiency and costs associated with raising poultry.

In Chapter 3, the suitability of a *Bacillus* based DFM to replace AGP was evaluated. Data from pen trials and a large commercial field trial clearly indicated that the DFM was a suitable candidate as a replacement to AGP, showing favorable results when compared to the most commonly used AGP (BMD) in conventional poultry production. The DFM consistently increased performance over BMD in all experiments, increasing BW by greater than 1.5% and an improvement in FCR of greater than 2.3%. DFM inclusion also showed strong performance benefits when incorporated into AGP free production. Growth rates of broiler chickens under existing industry conditions increased markedly. BW increased by 9.7% and FCR improved by 7.8%. When poultry producers achieve increases in FCR, the cost of goods is directly lowered. Feed costs contribute 65% of the total price of rearing poultry[7], amplifying the significance of improved FCR. Probiotics are well within the category of socially accepted performance enhancers, and seem to offer a strong solution to the pending quandary on how to replace AGP.

The second set of experiments, presented in Chapter 4 evaluated an OA product to determine if it would improve water consumption during pre-harvest feed withdrawal and reduce shrinkage during transportation from farm to processing plant. During the feed withdrawal period, water consumption decreases, which leads to welfare concerns and weight loss that has a significant impact on profitability. Previously published research from a large field trial in broilers indicated the impact of this commercially available OA product was significantly correlated to decreased transport associated losses, increasing profitability for the poultry company[8]. The findings in this study were similar when the product was applied in commercial turkeys, where OA-treated turkeys were 90 grams heavier than untreated turkeys after the feed withdrawal period. Additionally, shrinkage at the processing facility while waiting in the holding area was significantly reduced in turkeys that received OA during feed withdrawal. The OA product showed significant reduction in FW and transport associated losses, providing a strong financial return to the poultry producer.

Indications are that in these specific studies involving commercially available products, clear scientific evidence supports welfare and financial motivations to incorporate these select non-traditional technologies into poultry production programs. Both products are based on technologies that are commonly found in a variety of animal and human nutrition, and generally well accepted as safe by the public and government entities but have yet to be fully incorporated into industry standard practice. While they may not individually serve to effectively replace AGP and resolve poor management, presented evidence suggests that in production flocks, they can help improve production parameters of poultry. Due to the complex nature of rearing high-density flocks, it may be reasonable to assume that no single technology will have as great of an influence as AGP on the advancement of food animal production. However, combinations of alternative technologies may prove to effectively improve production parameters and well-being of the poultry and livestock industries and may possibly have additive or synergistic effects beyond those seen with AGP. Ongoing research will focus on combinations of such alternatives and further improvement of these existing products.

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